Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

(UGC recognized NAAC Re-accredited 'B++' grade state university)

"Bioethanol Production Using Algal and Agricultural Biomass"

Pre-Synopsis Submitted

For the Confirmation of the Ph.D. Topic

In the Subject

Microbiology Faculty of Science and Technology

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Introduction:

The world's conventional energy sources might not be able to meet up with the rising energy demands, as a result biofuels like bioethanol have emerged as possible substitute for the fossil fuels currently used in the transportation industry. Population growth and industrialisation constantly increasing hence global energy demand has consistently expanded over the past few decades. Ethanol has its major benefits on the minimization of carbon emissions when used as a fuel.[2] According to the International Energy Agency (IEA), the Global demand for biofuel would rise significantly by 2050. The increasing global warming and its impact on the environment due to CO2 emission can be minimised by using the bioenergy. One of the most promising biofuels is ethanol obtained from cellulosic biomass which obtained from microbial fermentation.

Cellulosic Biomass such as crop residues, grasses, woodchip, fruit bunch, trunk etc. are the potential feed stocks for production of bio ethanol. These raw materials are easily available in local areas throughout the world [23]. Cellulose is the most abundant Biomass in the world. Cellulose is a crystalline polymer of D- glucose residues connected by beta 1,4 glucosidic linkage being the primary structural material of plant cell wall. Therefore, it has become considerable economic interest to develop processes for effective treatment and utilisation of cellulosic waste as in expensive carbon source [22]. Cellulose is commonly degraded by an enzyme cellulase. This enzyme chiefly produced by fungi, bacteria and protozoans that convert the cellulose to cellobiose. Bacteria has high growth rate as compared to fungi they are good potential to be used in cellulase production. Bacterial genera such as *cellulomonas species, Pseudomonas species, Bacillus species*, has cellulolytic properties were reported. The Rapid rise in demand for alternative energy sources like bio ethanol has necessitated the use of fermentation techniques. The production of bioethanol from cellulosic biomass is a multifaceted research area that requires interdisciplinary approaches. Studies have demonstrated the potential of various feedstocks and technologies, yet significant strides are needed to overcome technical, economic, and sustainability barriers.

This research is intended to identify the suitable feedstock for bioethanol production from algal and agricultural cellulosic biomass and its utilities. In addition to this study will carry out to isolation, identification and characterization cellulose producing bacteria from its natural habitat and its ability to degrade cellulose to generate fermentable sugars which ultimately converts into bioethanol using natural yeast. This research will focus on greener and more sustainable energy for future.

Review of literature:

Studies on the Utilities of Different Cellulosic Biomass for the Production of Bioethanol

The production of bioethanol from cellulosic biomass has been a subject of extensive research due to its potential to replace fossil fuels and reduce greenhouse gas emissions. This review explores significant contributions and developments in the field, emphasizing the characteristics, pre-treatment methods, enzymatic hydrolysis, fermentation strategies, and comparative assessments of various cellulosic feedstocks.

1. Lignocellulosic Biomass Composition and Potential

Lignocellulosic biomass is composed of three primary components: cellulose, hemicellulose, and lignin. Cellulose (35–50%) is a polymer of glucose, hemicellulose (20–35%) contains heterogeneous sugars, and lignin (10–25%) provides structural rigidity. Research has highlighted the following aspects

Agricultural Residues: Studies suggest that rice straw, wheat straw, and corn stover, demonstrating their abundance and high cellulose content [25].

Energy Crops: Switchgrass and miscanthus have been identified as promising feedstocks due to their rapid growth and high biomass yield [13].

Forestry Waste: Sawdust and wood chips offer regional advantages, though higher lignin content often complicates conversion processes [26].

2. Pre-treatment Technologies :

The pre-treatment stage is crucial to disrupt the recalcitrant structure of lignocellulosic biomass, enhancing enzymatic accessibility to cellulose. Different pre-treatment methods have been studied:

Chemical Pre-treatment:

Acid hydrolysis has been widely used for biomass like corn Stover and rice straw, although issues like sugar degradation persist [5].

Alkaline pre-treatment, effective for delignification, has shown promising results in treating energy crops [16].

Physical Methods:

Techniques such as ball milling and steam explosion improve biomass digestibility but may require high energy input [10].

Biological Pre-treatment:

Fungi-mediated delignification has been reported to be environmentally friendly, though it often requires longer reaction [24].

Recent studies emphasize the integration of multiple pre-treatment methods to balance efficiency and cost [8].

3. Enzymatic Hydrolysis

Enzymatic hydrolysis is a critical step in converting pre-treated biomass into fermentable sugars.

Key findings include:

The efficiency of hydrolysis depends on enzyme loading, biomass structure, and reaction conditions. *Trichoderma reesei*-derived cellulases are widely used, but their activity is often inhibited by lignin.

Studies have explored the use of accessory enzymes such as xylanases to enhance hemicellulose breakdown [15].

Advancements in enzyme engineering have produced thermostable and high-specificity cellulases, improving sugar yields from biomass like wheat straw [12].

4. Fermentation Technologies:

The conversion of sugars to ethanol is typically achieved using microbial fermentation. Key findings in this domain are:

Microbial Strains:

Saccharomyces cerevisiae is the most common organism used for glucose fermentation but struggles with pentose sugars from hemicellulose.

Recombinant strains, such as engineered *Escherichia coli* and *Zymomonas mobilis*, have shown improved capabilities to ferment both hexose and pentose sugars [11].

Consolidated Bioprocessing (CBP) :

CBP integrates enzyme production, hydrolysis, and fermentation into a single step. Studies on Clostridium thermocellum indicate its potential for high-yield ethanol production [12].

Process Optimization:

Simultaneous saccharification and fermentation (SSF) reduces enzyme inhibition and energy costs compared to separate hydrolysis and fermentation (SHF) [5].

5. Comparative Studies on Biomass Feedstocks :

Comparative studies have evaluated the utilities of different feedstocks based on their chemical composition, sugar yield, and conversion efficiency:

Rice Straw and Wheat Straw: Both have high cellulose content (~40%), but rice straw's higher silica content can pose challenges during pretreatment [7].

Corn Stover: Studies highlight its high fermentable sugar yield after alkaline pretreatment, making it one of the most studied feedstocks [12].

Switchgrass and Miscanthus: Energy crops demonstrate higher yields per hectare compared to agricultural residues and have lower seasonal variability [13].

6. Economic and Environmental Considerations :

Life Cycle Assessment (LCA): LCAs on cellulosic ethanol production reveal significant reductions in greenhouse gas emissions compared to fossil fuels, with emissions varying based on feedstock type and location [10].

Techno-Economic Analysis: Challenges such as high enzyme costs, energy-intensive pretreatments, and logistics of biomass collection and storage remain critical for scaling up production [14]

Research gap

- Lack of a comprehensive database comparing diverse regional feedstocks under standardized conditions.
- Need for further development of low-cost, environmentally sustainable pretreatment methods.
- Enhanced microbial strains capable of efficiently fermenting mixed sugar streams.
- Integration of bioethanol production with bio refineries to utilize lignin for value-added products and energy recovery.
- Emerging technologies like artificial intelligence in process optimization, and advancements in bioreactors are likely to address these challenges in the near future.

Aims and objectives

Present study undertaken with following aims and objective:

- Isolation and characterization of cellulase producing microbes from natural habitat.
- Optimization of Cultural conditions.
- Assay of cellulase enzyme.
- Determination of cellulose contains of different cellulosic Biomass like agricultural and aquatic.
- Determination and detection of fermentable sugar in enzymetically treated cellulosic Biomass.
- Fermentation of cellulosic biomass with yeast to produce ethanol.
- Detection and characterization of ethanol.

Materials and methods

1. Biomass Collection and Preparation

Aquatic Biomass: Collect *Eichhornia crassipes* (water hyacinth), algae, or similar aquatic weeds from local water bodies. Wash, sun-dry, and grind to a uniform particle size (1–2 mm) for further analysis[11].

Agricultural Biomass: Source rice straw, wheat straw, corn stover, or sugarcane bagasse from nearby farms. Air-dry and grind to a similar size. Store in airtight containers to prevent moisture absorption[11].

2.Pretreatment of Biomass

Chemical Pretreatment: Treat aquatic biomass with 2% NaOH at 80°C for 1 hour to reduce lignin content. For agricultural biomass, use 1% H2SO4 at 120°C for 30 minutes to hydrolyze hemicellulose[11].

Physical Pretreatment: Apply steam explosion (180–200°C) to disrupt lignocellulosic structures, enhancing enzymatic accessibility[1].

Biological Pretreatment: Use white-rot fungi or ligninolytic enzymes (laccase, manganese peroxidase) to degrade lignin sustainably.

3. Isolation of Native Microbial Flora

Source: Collect decomposing biomass or soil from waterlogged fields.

Enrichment: Culture microorganisms in carboxymethyl cellulose (CMC) broth at 30°C to enrich cellulolytic strains.

Screening: Plate isolates on CMC agar and identify cellulase producers using Congo red staining. Measure enzyme activity (cellulase and xylanase) using standard assays[11][1].

4. Enzymatic Hydrolysis

Procedure: Incubate pretreated biomass with crude or purified enzymes produced by isolated native strains. Optimize hydrolysis conditions (pH ~5, temperature 50°C, 24–48 hours).

Analysis: Quantify released reducing sugars using the DNS (3,5-dinitrosalicylic acid) method[11].

5. Fermentation Process

Inoculum Preparation: Develop inoculum using isolated yeast strains (e.g., *Saccharomyces cerevisiae*) from local flora.

Fermentation: Ferment the hydrolysates anaerobically at 30–35°C for 72 hours. Measure ethanol yield using gas chromatography or high-performance liquid chromatography (HPLC)[1].

Co-Culture Strategy: Use mixed microbial cultures for simultaneous utilization of hexose and pentose sugars[1].

6. Process Optimization

Employ Design of Experiments (DoE) and Response Surface Methodology (RSM) to optimize key parameters such as enzyme concentration, temperature, and fermentation time[1].

7. Product Analysis

Ethanol Yield: Calculate ethanol yield as a percentage of theoretical yield based on sugar content.

Byproducts: Analyze lignin residues and unfermented sugars to assess process efficiency.

8. Environmental and Economic Assessment

Conduct life cycle analysis (LCA) to evaluate energy use and greenhouse gas emissions. Perform a techno-economic analysis to assess cost-effectiveness.

Proposed work plan

Year I:

Literature Review and Experimental Setup

Comprehensive review of aquatic and agricultural cellulosic biomass for bioethanol production.

Analyze existing technologies, focusing on native microbial flora for enzymatic hydrolysis and fermentation.

Sample Collection and Characterization.

Collect aquatic biomass (e.g., algae, water hyacinth) and agricultural residues (e.g., rice straw, corn stover).

Perform proximate analysis, structural characterization, and compositional analysis of cellulose, hemicellulose, and lignin.

Establishment of Experimental Protocols

Design experimental setup for pretreatment, hydrolysis, and fermentation.

Optimize screening protocols for isolating native microbial strains.

Year II:

Isolation and Optimization of Native Microbial Flora

Microbial Isolation and Screening

Isolate cellulose-degrading microbes from local environments (e.g., soil, decomposing biomass).

Screen microbes for cellulase, xylanase, and ligninase activities.

Enzymatic Hydrolysis Studies

Evaluate hydrolysis efficiency using native microbial enzymes on pretreated biomass.

Optimize enzymatic conditions (e.g., pH, temperature, incubation time).

Preliminary Fermentation Studies

Test ethanol production using hydrolysates and isolated native microbial flora.

Monitor sugar utilization and ethanol yield.

Year III:

Process Optimization and Scale-Up Studies

Pretreatment Optimization

Optimize pretreatment techniques for maximum cellulose availability.

Evaluate different combinations of physical, chemical, and biological pretreatments.

Fermentation Optimization

Optimize fermentation parameters (temperature, pH, microbial load).

Explore co-culture strategies for efficient hexose and pentose sugar utilization.

Perform process simulations for energy and cost efficiency.

Publish results in peer-reviewed journals.

Complete PhD thesis with comprehensive findings, conclusions, and future perspectives.

This structured plan ensures a systematic approach to addressing all aspects of the research, from basic studies to applied solutions for bioethanol production.

Target to be achieved

- This research will contribute to advancing knowledge in the field of bio ethanol by
- Identifying the most efficient and sustainable cellulosic Biomass feed stock.
- Developing optimised conversion processes tailored to specific Biomass types.
- Biomass utilise using microbes to produce ethanol.
- Supporting Global efforts to achieve sustainable development goals.

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